Application of *In Vitro* Toxicogenomics Towards Drug Safety Evaluation

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Group Leader, Toxicogenomics
Abbott Laboratories

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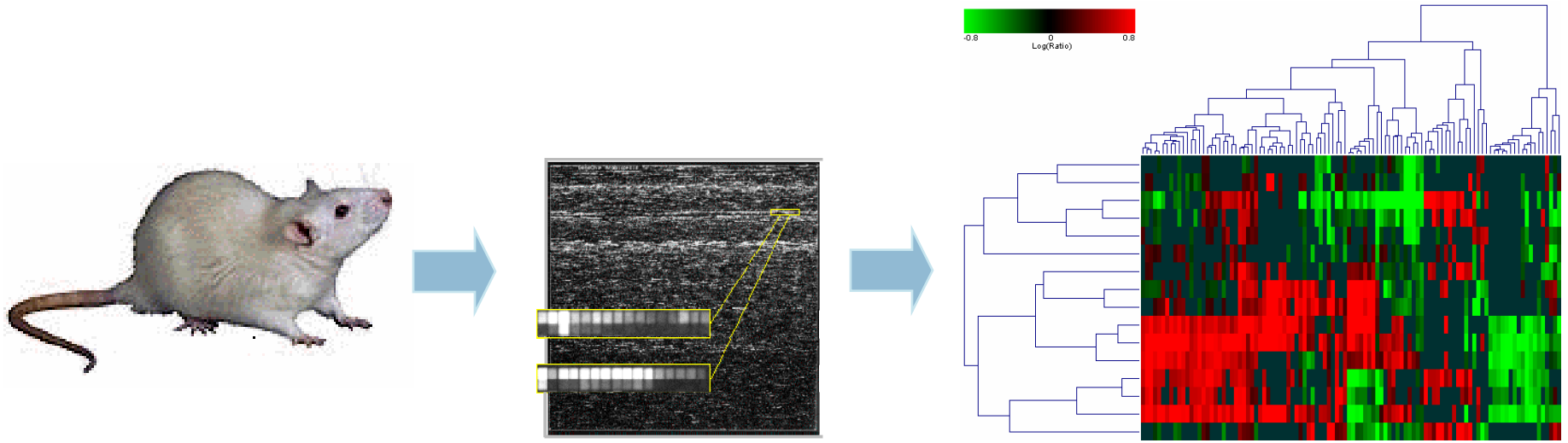
Toxicogenomics

The application of gene expression analysis systems towards drug safety evaluation



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Information Gained from Toxicogenomics

- Patterns of gene expression changes associated with toxicity and with potential predictive value
- Specific gene expression changes related to the mechanism of toxicity
- Gene expression changes that can be used to bridge animal and human safety studies

Toxicological Characterization in Discovery

***In Vitro* Ideal**

1 day
of dosing
mg
of compound

Results in
days

Animal Studies

weeks
of dosing

100s-1000s
grams of drug

Results in 2-3
months

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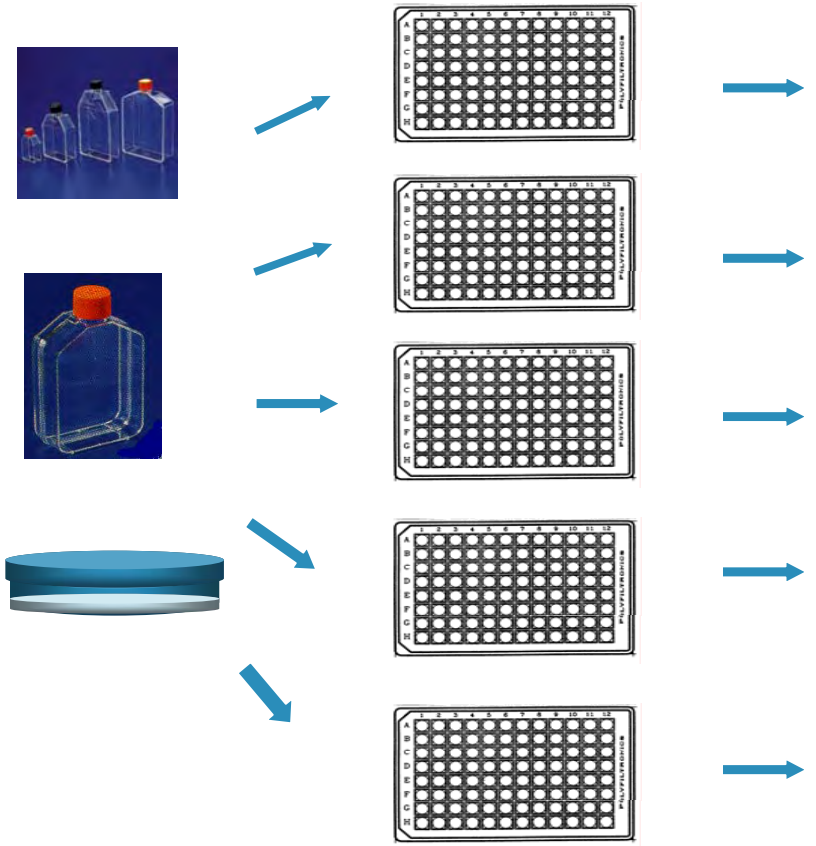
Traditional *In Vitro* Toxicology Paradigm



- Cytotoxicity
  - –MTT
  - –LDH
- Mitochondrial Damage
  - –Mitochondrial respiration
  - –Mitochondrial permeability transition
- Oxidative Stress
  - –GSH depletion
  - –ATP
- Apoptosis
  - –Tunnel
  - –Caspase
- Steatosis
  - –Nile red
- Phospholipidosis
  - –NBD-PE staining of hepatocytes

Several Cell Types

Several Doses
Several Assays
Lots of Reagents

Data points: +++++
Interpretation: ????

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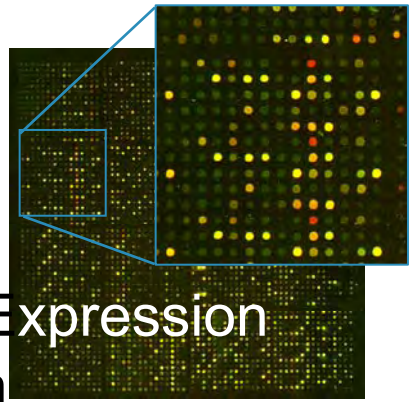
The *In Vitro* Toxicogenomics Paradigm

Collaboration with Iconix Pharmaceuticals



Gene Expression Pattern

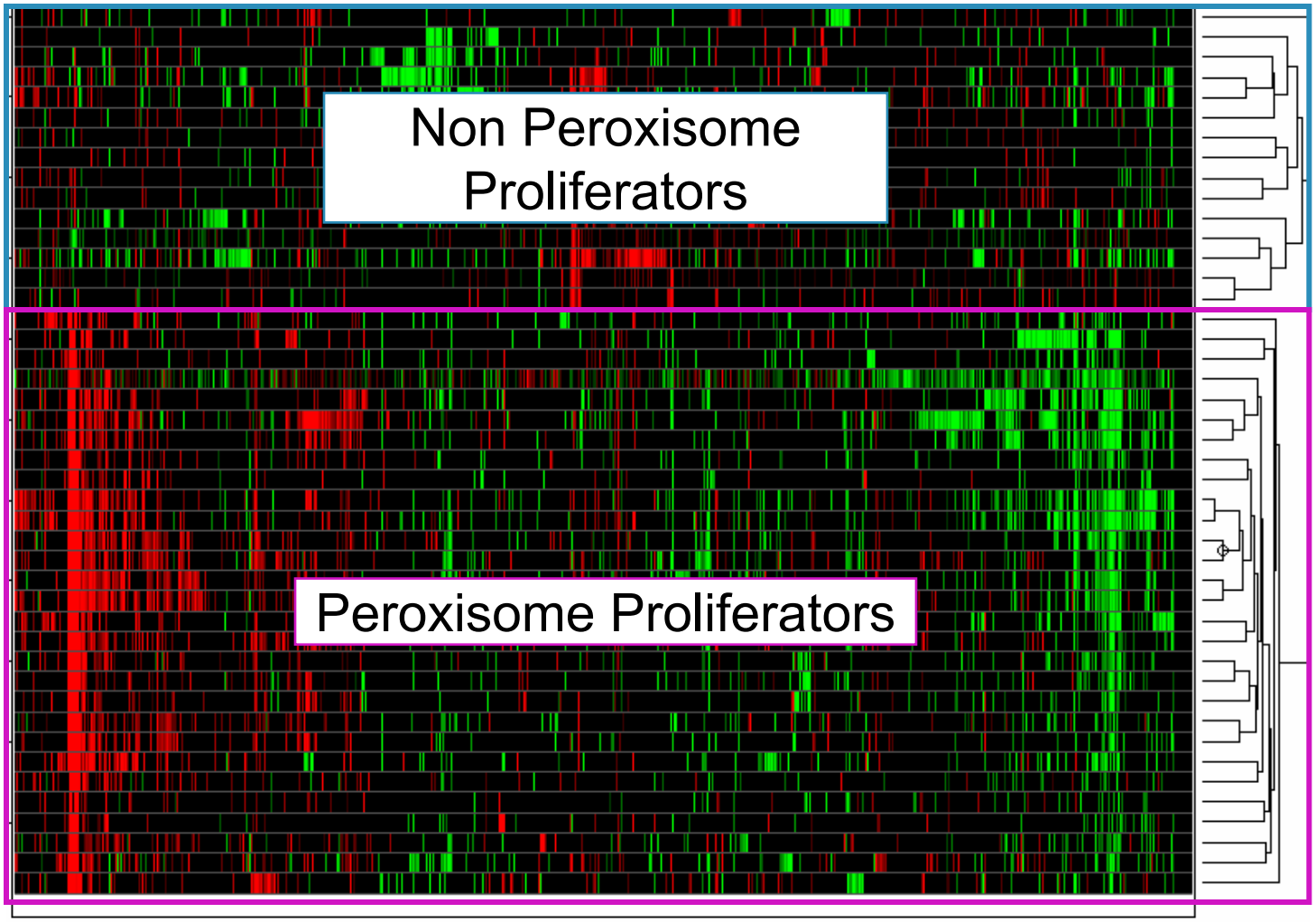
- Apoptosis
- Necrosis
- Canalicular cholestasis
- Microvesicular steatosis
- Peroxisome proliferation
- Ah-receptor agonist
- Phospholipidosis

One Cell Type

One Dose
One Assay
One Reagent Type

Data points: Limited
Interpretation: Simple

Mechanistically Similar Toxicants Induce Similar Gene Expression Changes *In Vivo*



Contains data from Iconix DrugMatrix™

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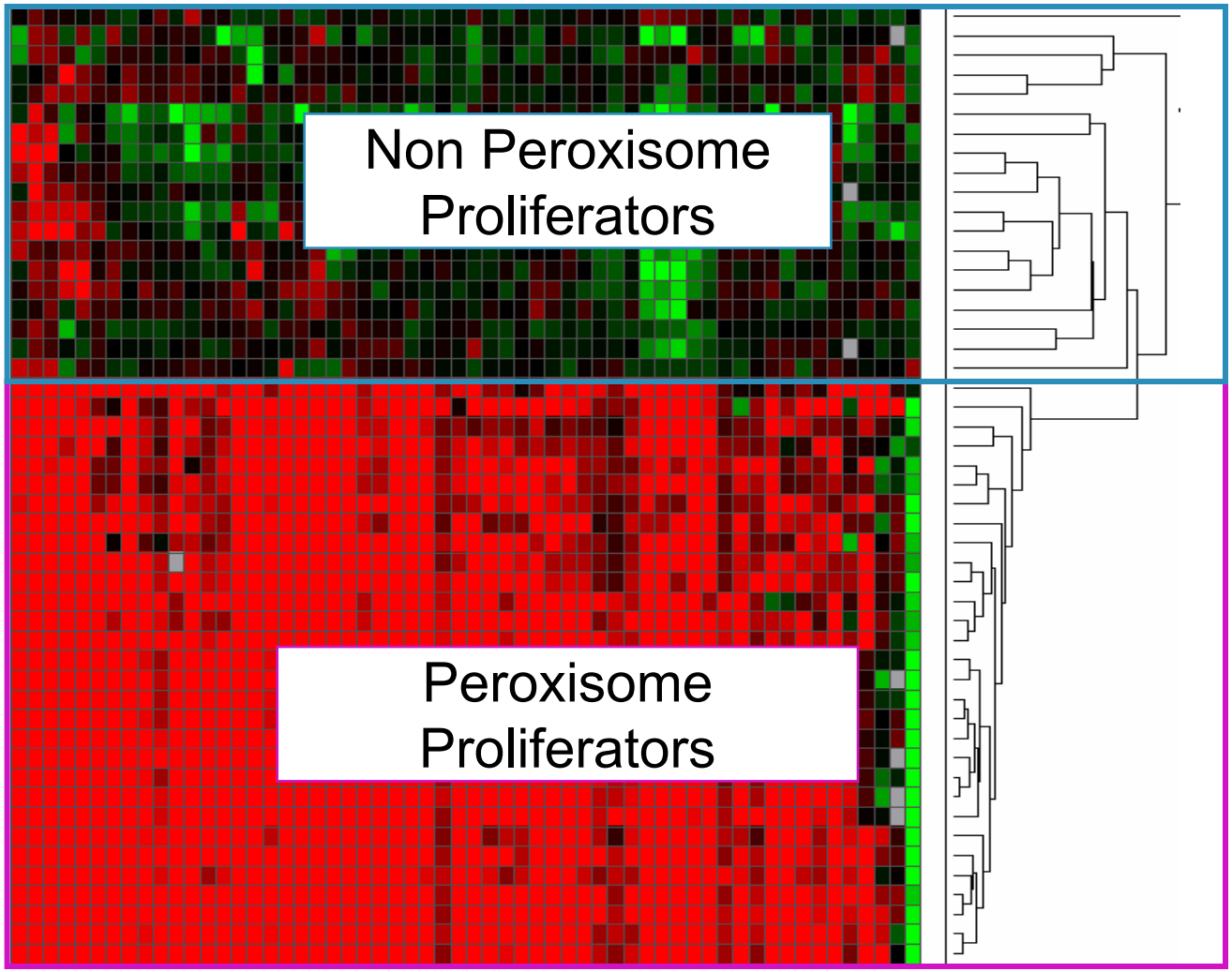
Signatures Can Be Generated for Mechanistic Class *In Vivo*

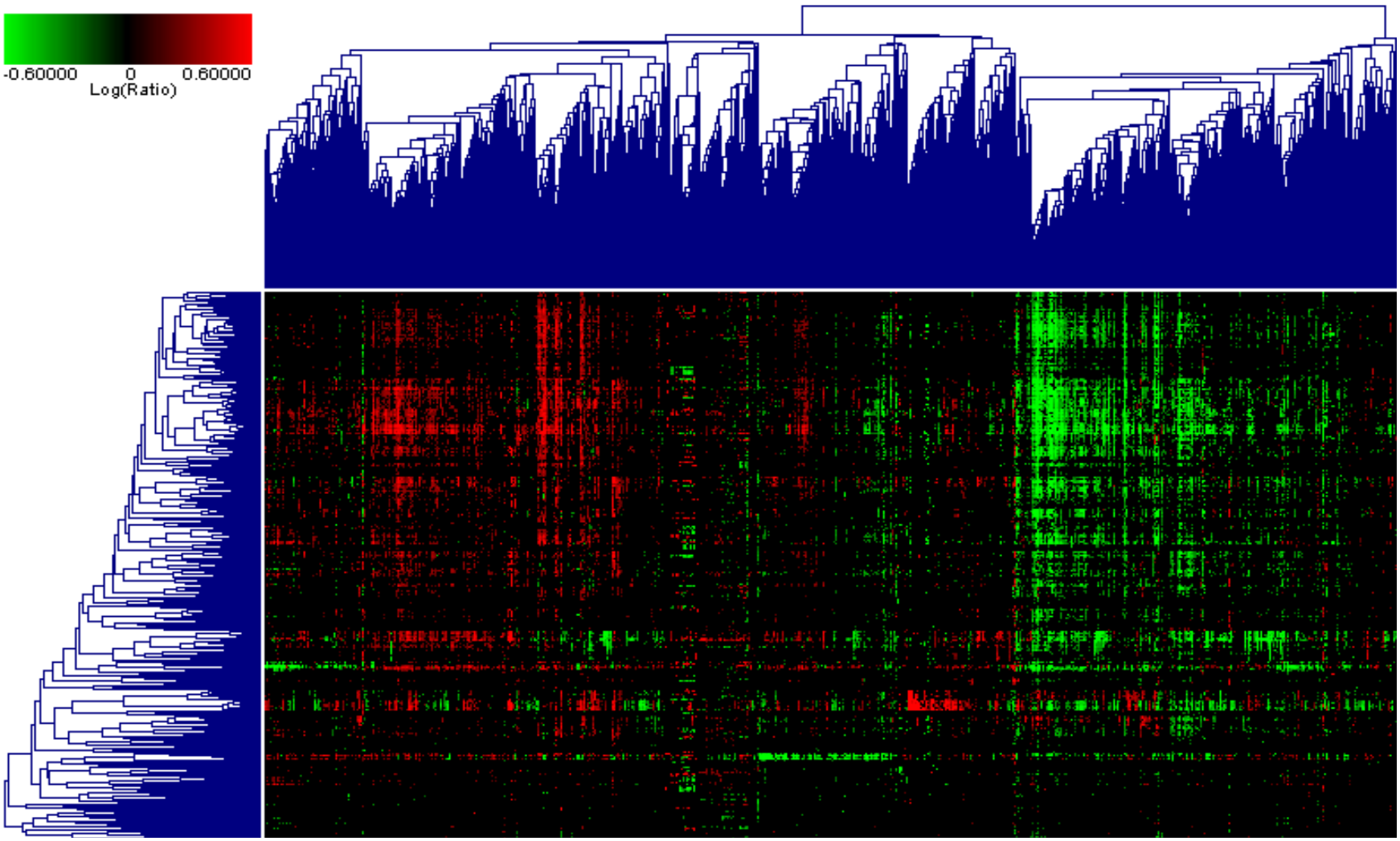


Contains data from Iconix DrugMatrix™

Toxicogenomics *in vitro* Assays: Rat Hepatocyte Protocol

- Isolated rat hepatocytes cultured for 24 hours before treatment
- Cells treated for 24 hours with compound at TC20 concentration
- 3 isolations used for all compounds
- Hepatocytes treated with compounds that are prototypical inducers of the toxicity
- Signatures created by identifying similar gene expression changes caused by compounds in the same mechanistic class
- 45 reference compounds
- 15 validation compounds
- 40 negative control compounds

Expression Profiles in Primary Rat Hepatocytes



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In Vitro Toxicogenomics

- Do compounds with similar mechanisms of toxicity give similar expression profiles *in vitro*?
- Can gene sets or signatures be identified that can be used to screen compounds?
- Can signatures be used to classify new compounds?
- What concentration should be used to screen new compounds?
- Can toxicogenomics be performed using assays that are more high throughput?
- How should these data be used for compound selection in drug discovery?

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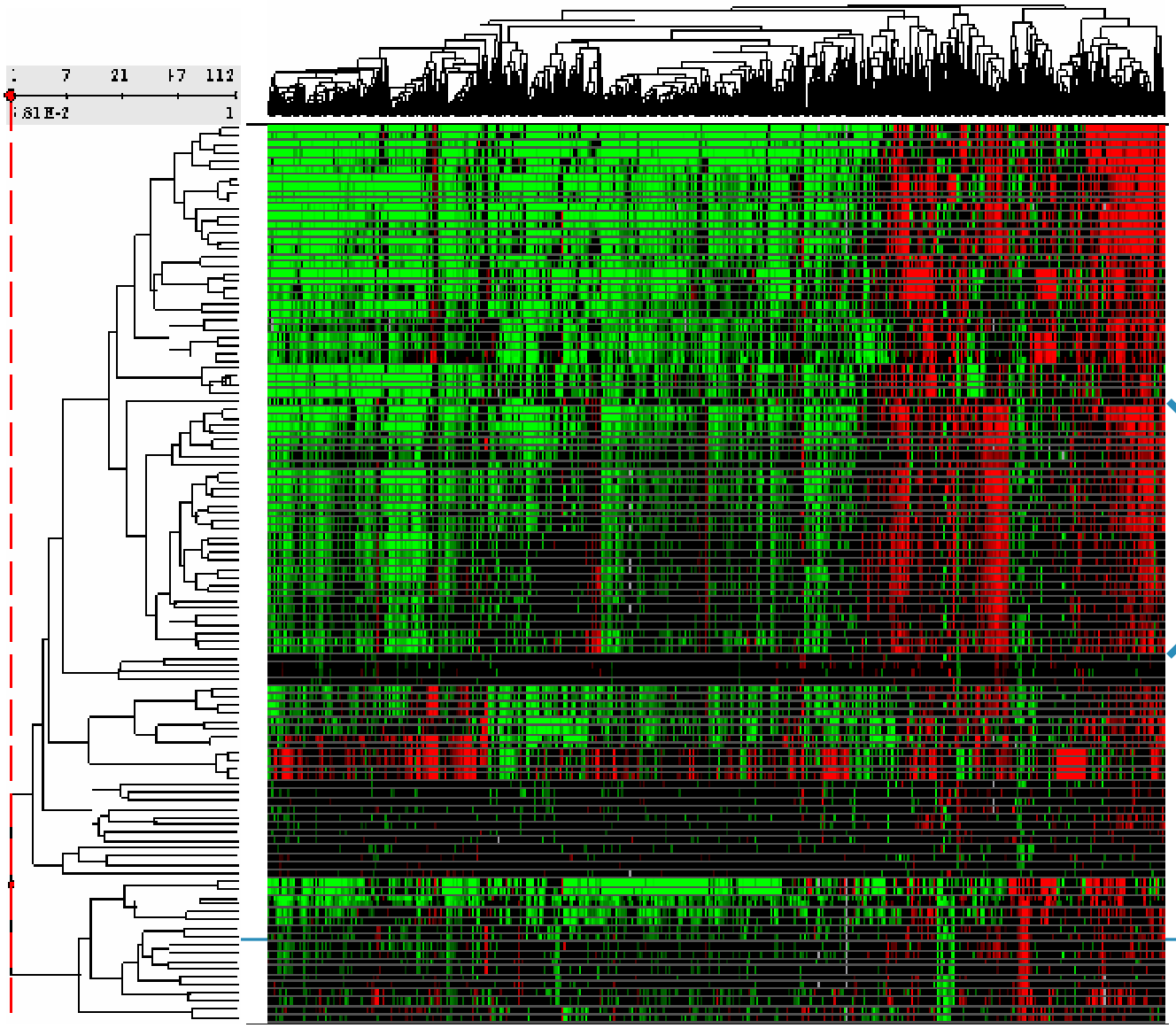

Phospholipidosis Compounds

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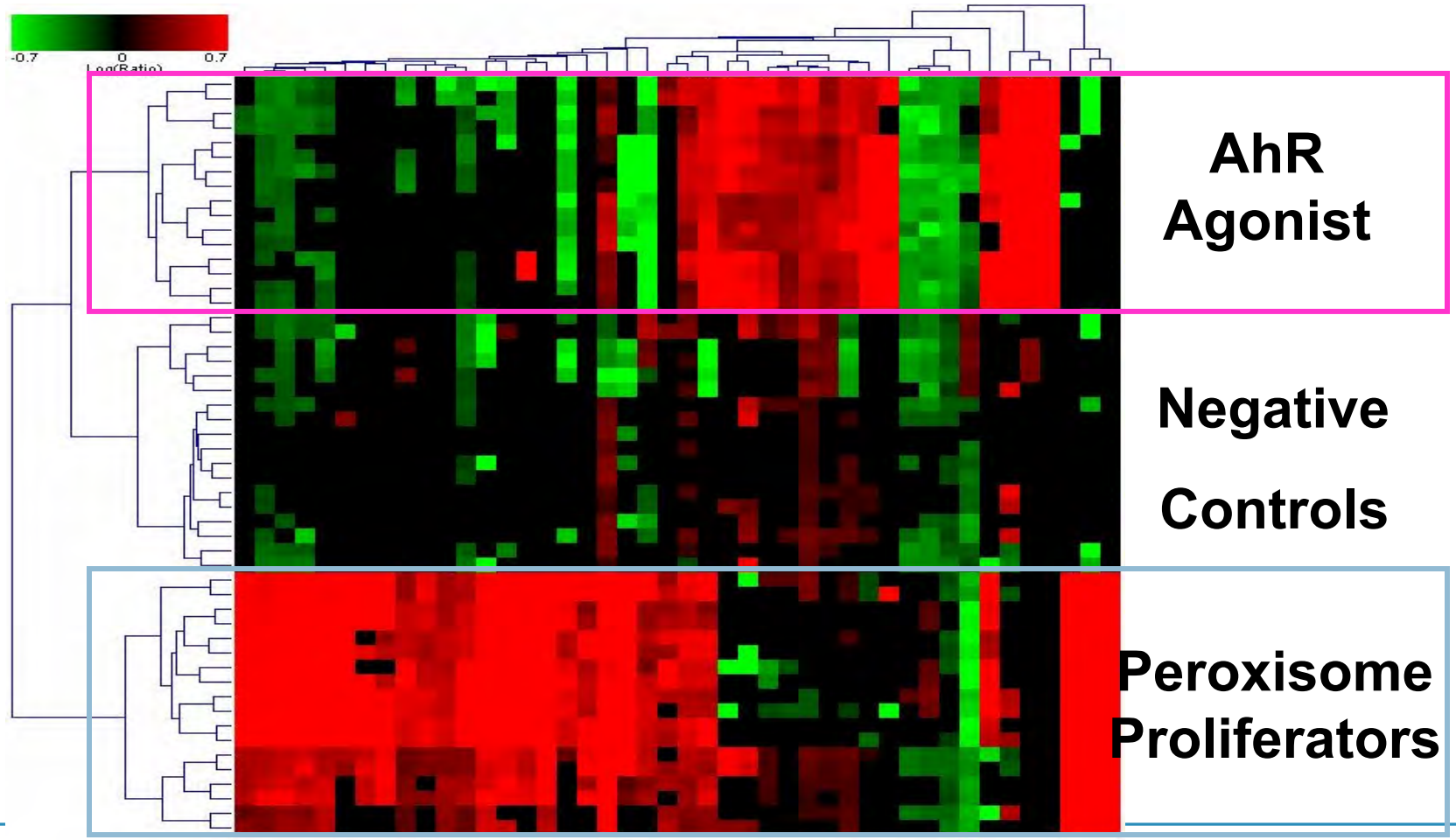
Compounds Tested for *In Vitro* Toxicogenomics

Compound Classes	Compounds
AhR agonist	**3MC, Aroclor, Beta Napthoflavone**
Peroxisome Proliferator	**Clofibrate, Bezafibrate, WY-14643**
Negative Control	**Penicillin, Spectinomycin, Chlorpheniramine**

Compounds Classification using Hierarchical Clustering Analysis



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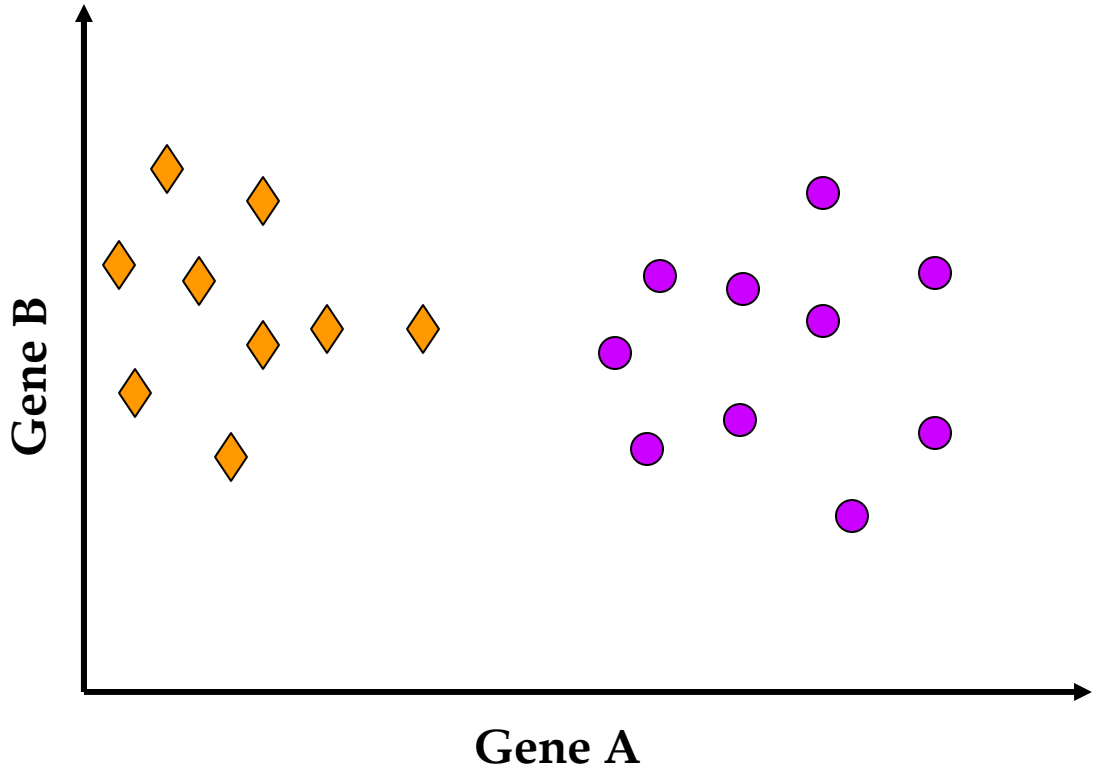
Linear Discriminant Analysis (LDA): a Statistical Algorithm for Classification



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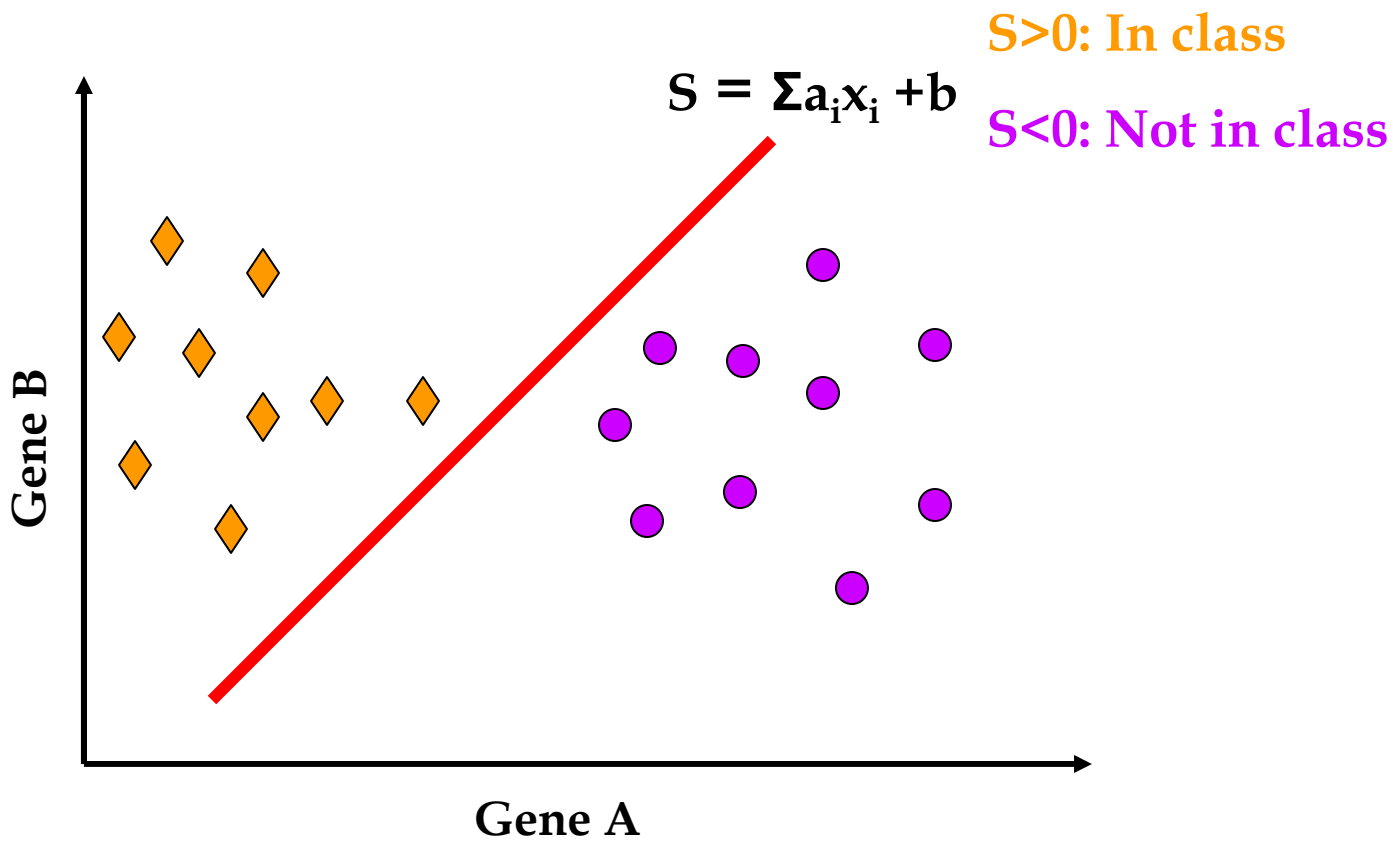

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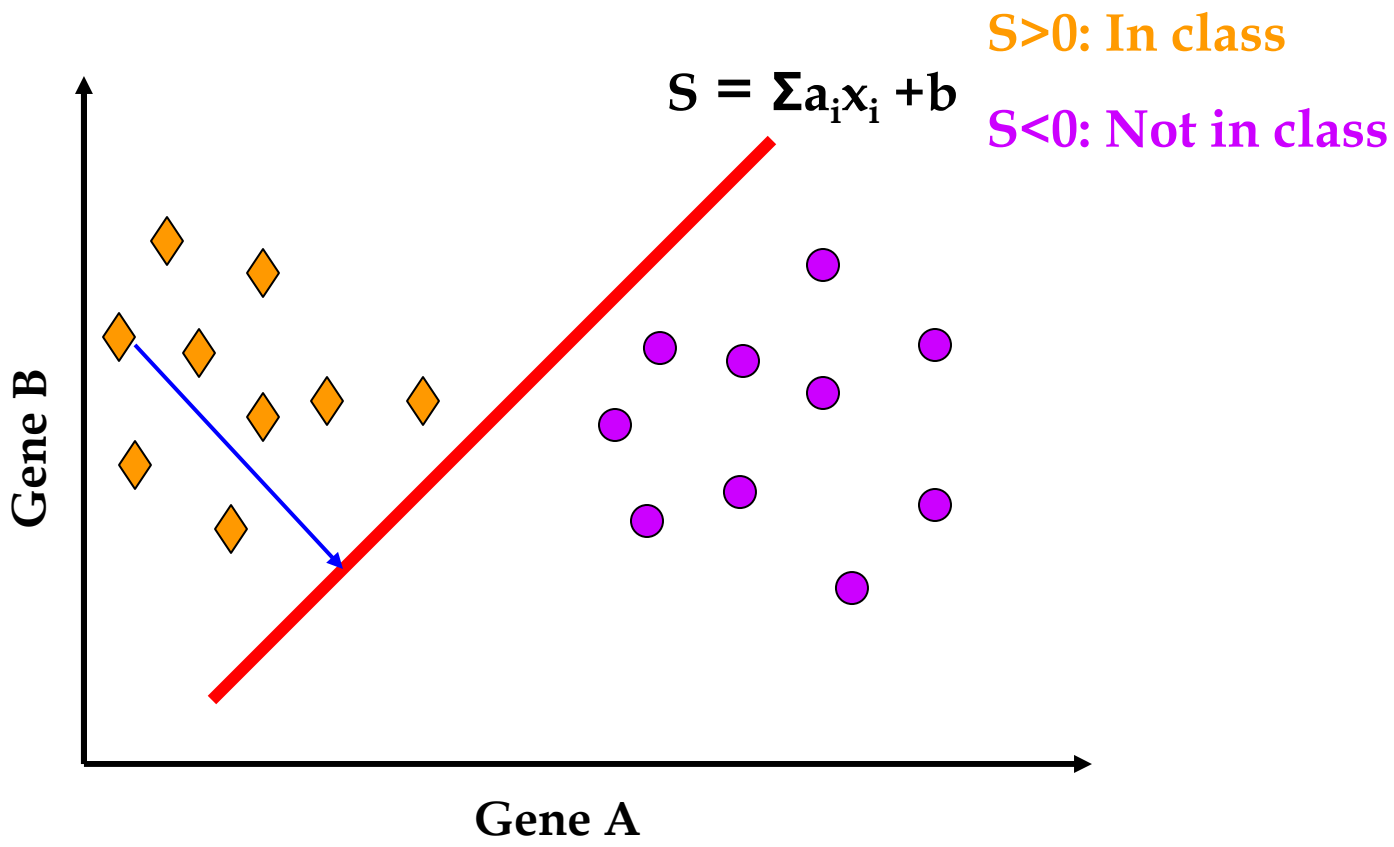

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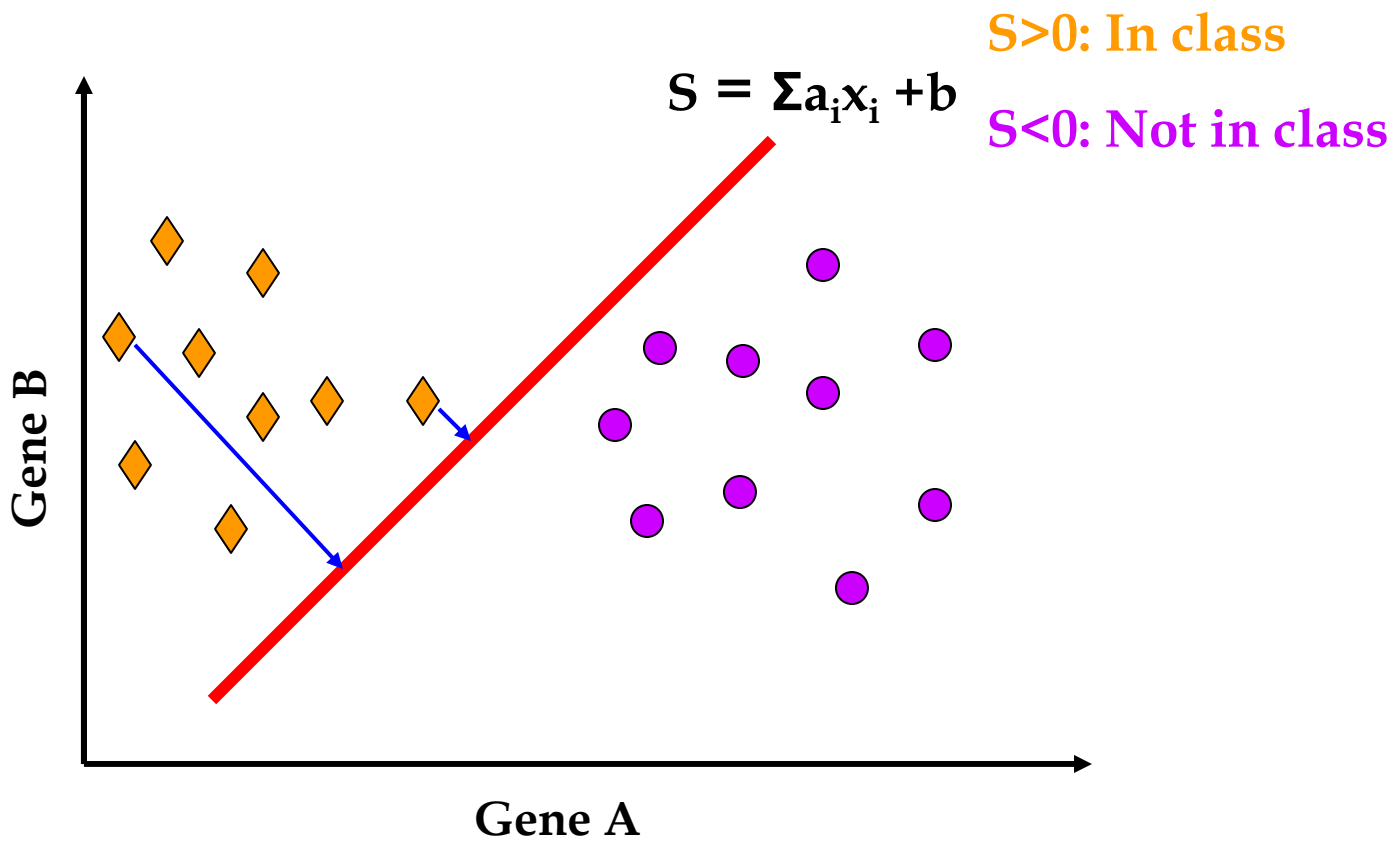

In Vitro Toxicogenomics

- Do compounds with similar mechanisms of toxicity give similar expression profiles *in vitro*?
- Can gene sets or signature be identified that can be used to screen compounds?
- **Can signatures be used to classify new compounds?**
- What concentration should be used to screen new compounds?
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- How should these data be used for compound selection in drug discovery?

Validation Compounds for *In Vitro* Signatures

- **AhR Agonist**
  - Benzo(a)pyrene
  - A-277249
  - Omeprazole
- **Peroxisome Proliferator**
  - Fenoprofen
  - Indomethacin
- **Negatives**
  - 3',3-diindolylmethane (DIM)
  - Troglitazone

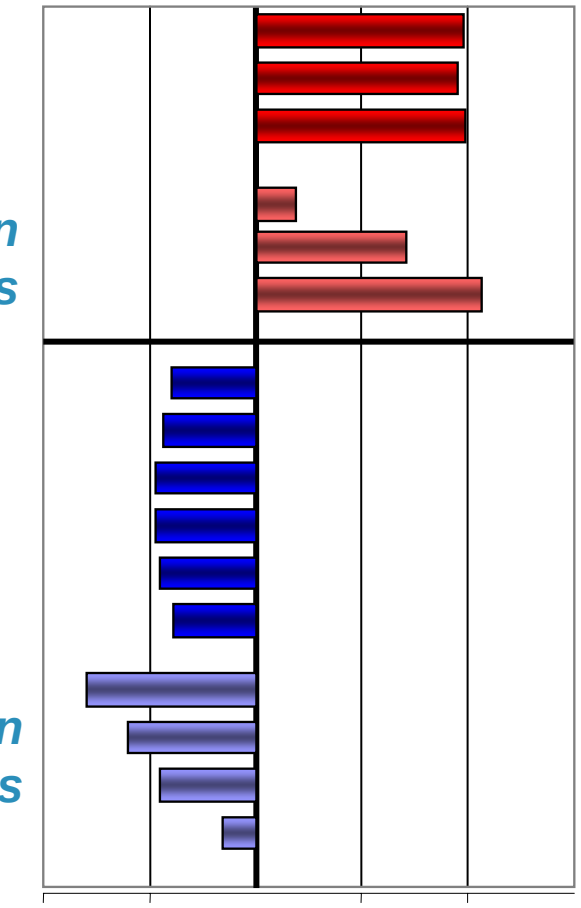
In Vitro Signatures Correctly Classify Known Hepatotoxins

Ah Receptor Activators

Validation Compounds

Control Compounds

Validation Compounds



3MC, 100 µM
Aroclor, 100 µM
Napthaflavone, 75 µM

Omeprazole, 300 uM
Benzo (a) pyrene, 10 µM
A-277249, 108 µM

Chlorpheniramine, 275 µM
Penicillin, 150 µM
Spectinomycin, 90 µM
Bezafibrate, 200 µM
Clofibrate, 550 µM
WY-14643, 35 µM

Fenoprofen, 300 µM
Troglitazone, 75 µM
Indomethacin, 235 µM
3,3' diindolylmethane, 75 µM

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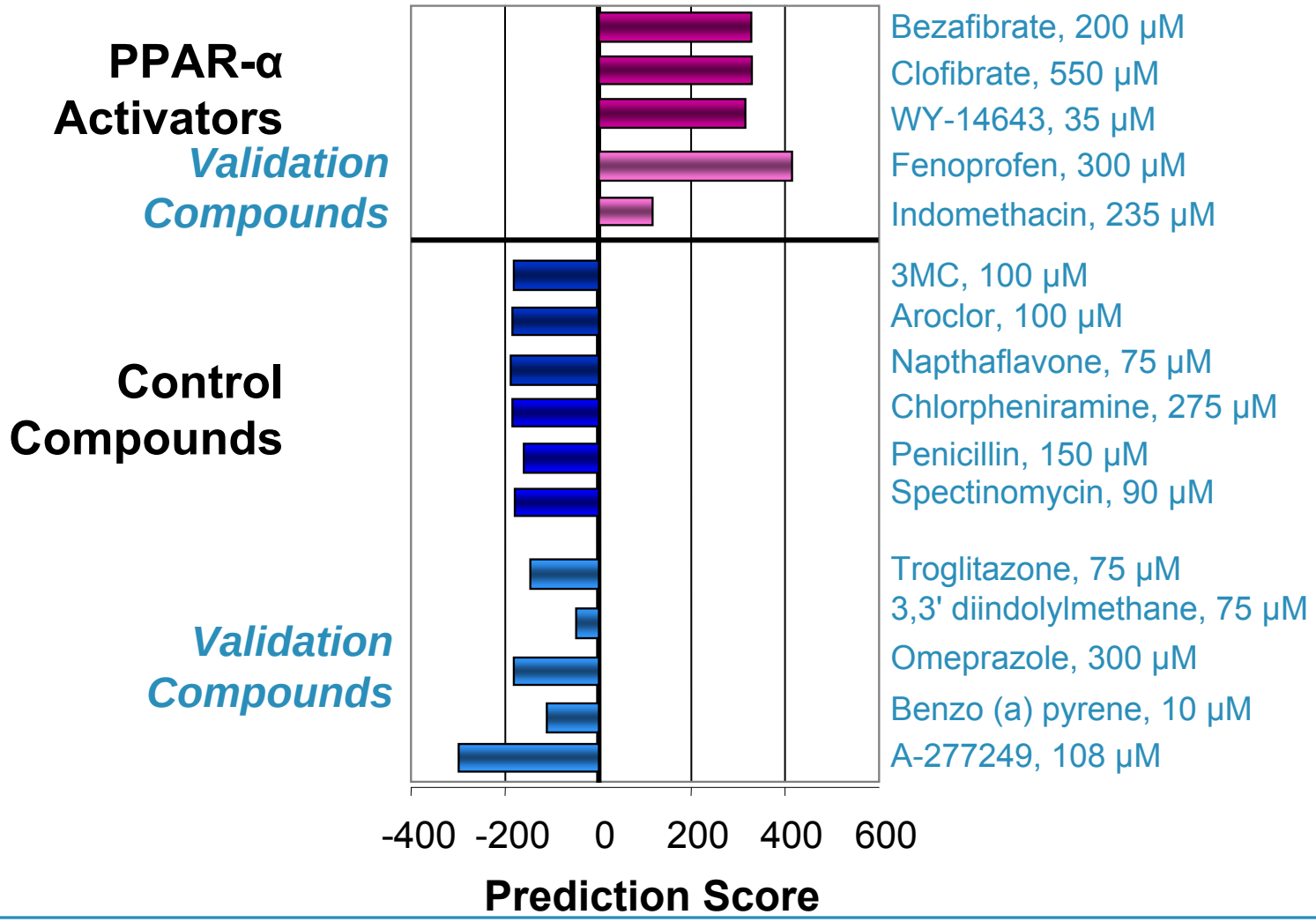
In Vitro Signatures Correctly Classify Known Hepatotoxins



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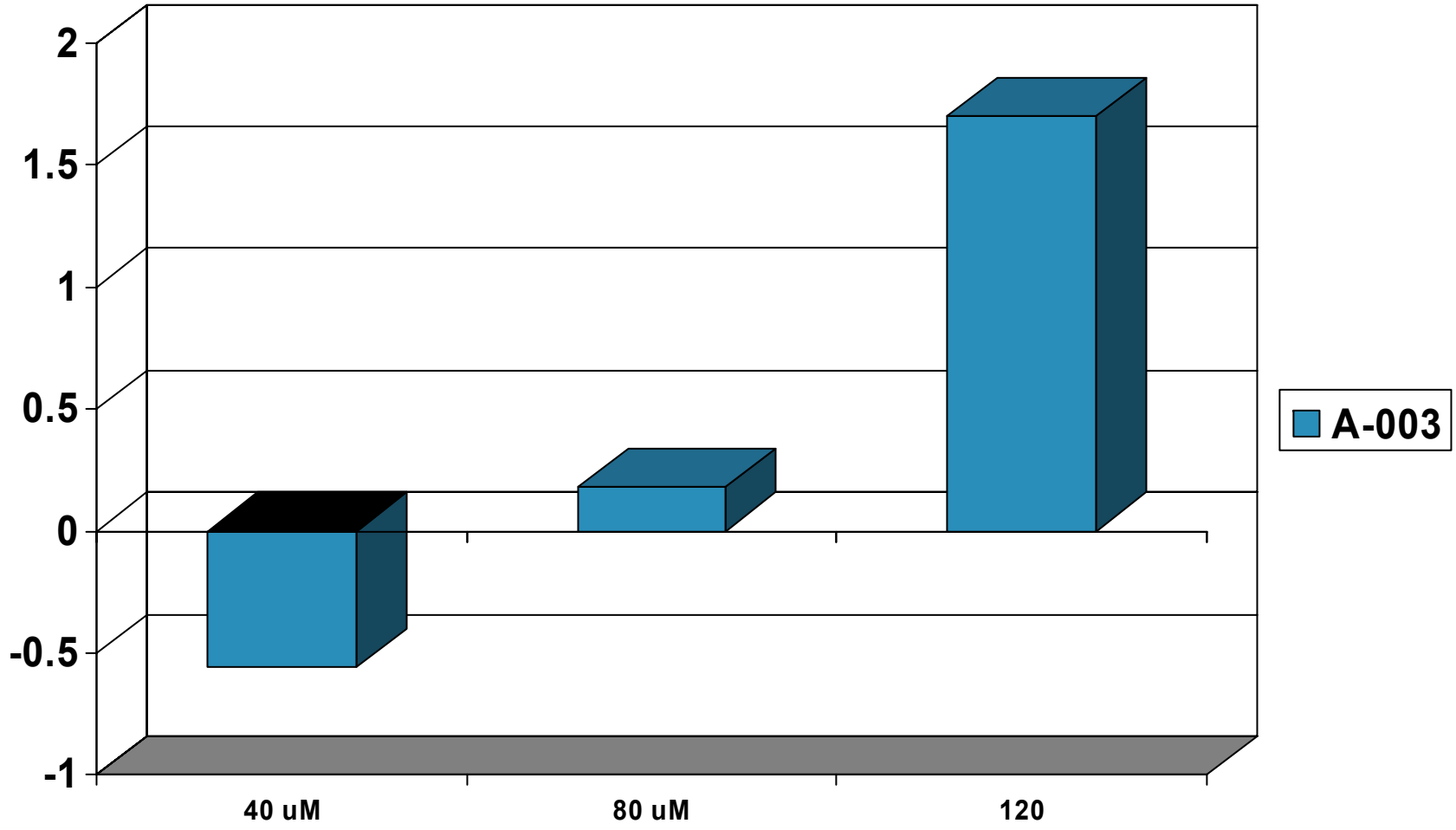
Phospholipidosis Signature

Compound	Dose	Result in Phospho. Cell Assay	Phospholipidosis Signature
A-001	40 μM	+	+
A-002	40 μM	++	++
A-003	40 μM	+	-
Cyclophosphamide	1.32 mM#	NA	-
Doxorubicin	1.5 μM #	NA	-
Methapyrilene	300 μM #	NA	-
Rifampin	125 μM #	NA	-

#: dose corresponding to TC20 at 24 hr.

Phospholipidosis Signature



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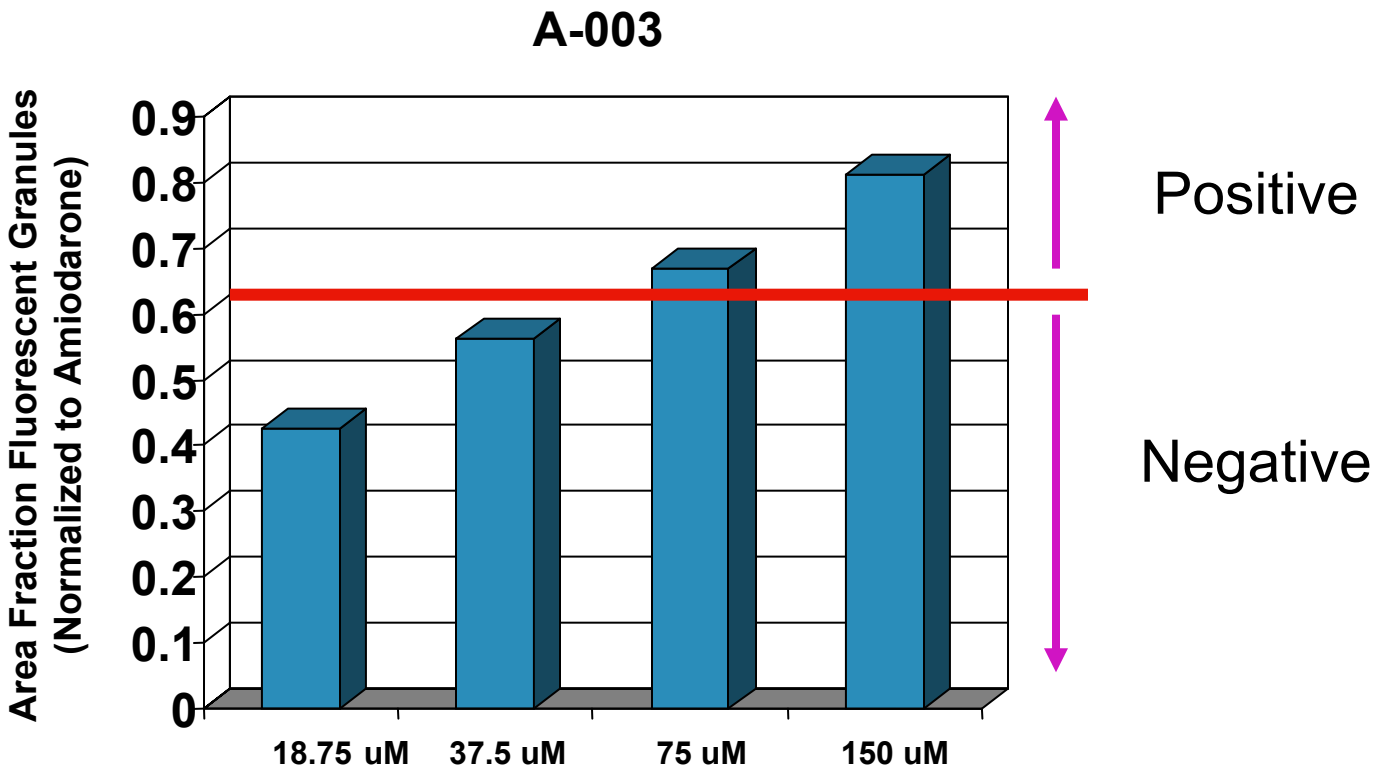
Phospholipidosis Cell Based Assay



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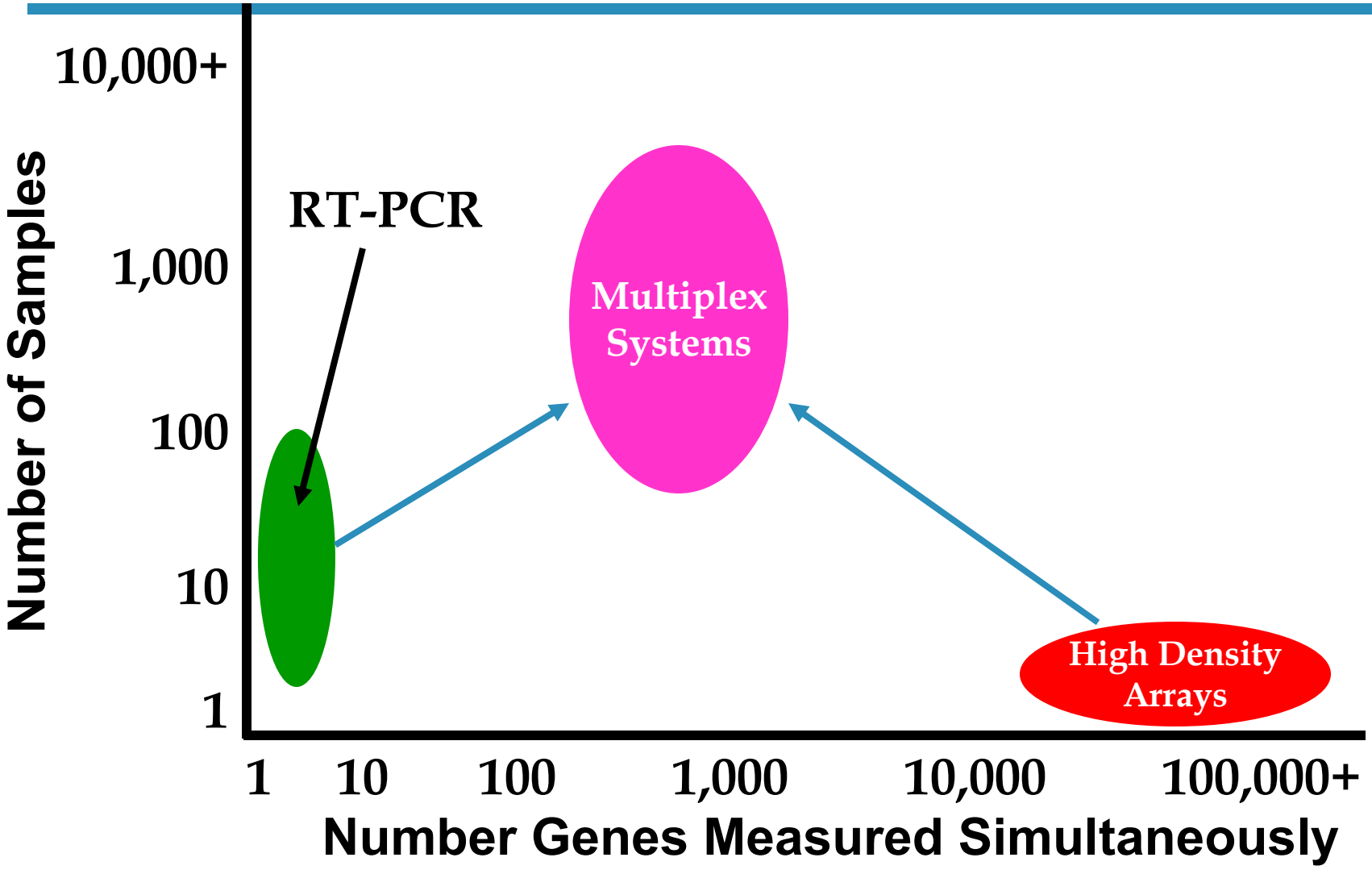
Gene Expression Profiling:
Moving Toward Higher Throughput



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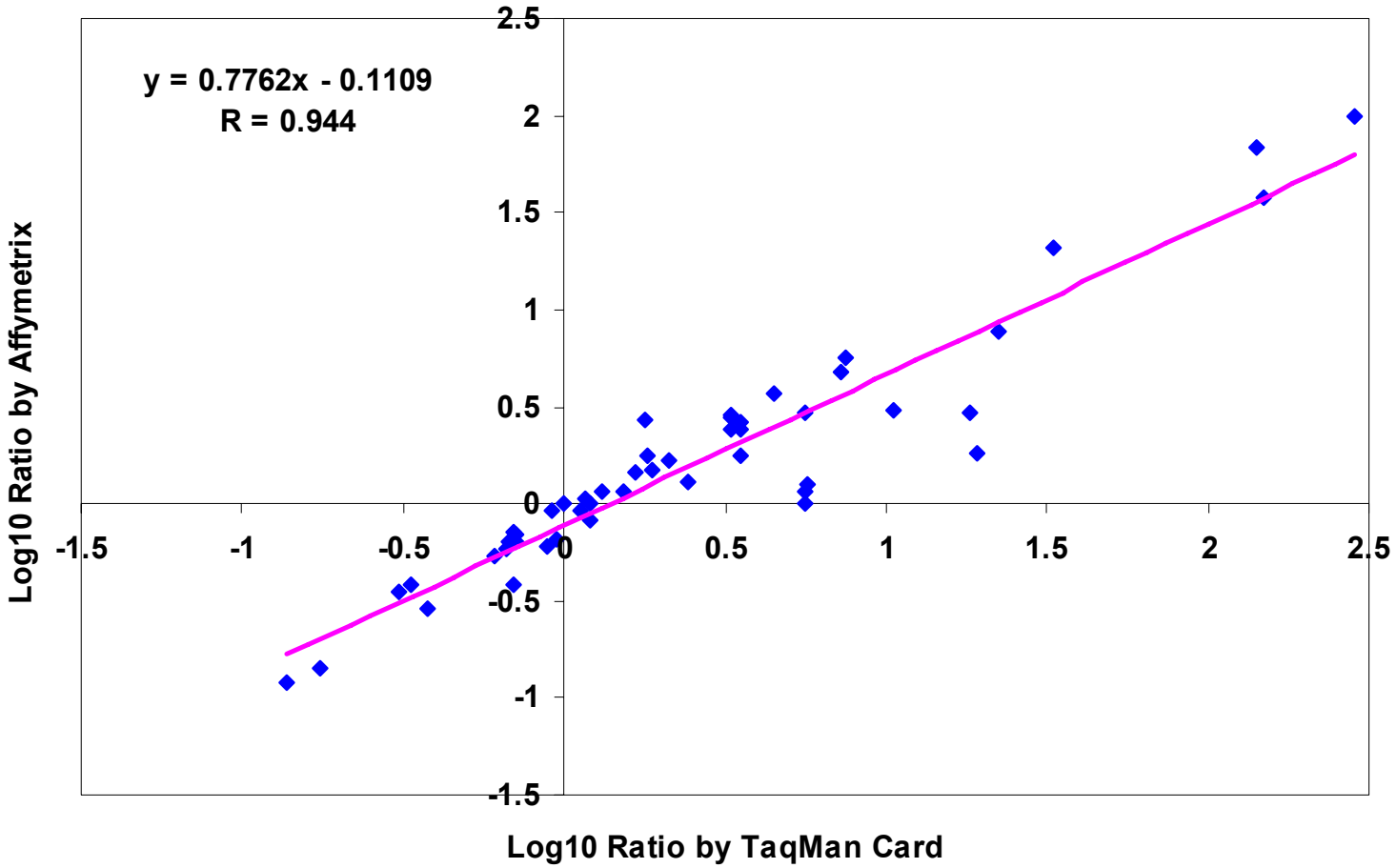
TaqMan Micro Fluidic Card

- Capable of identifying expression changes up to 200 genes
- Ability to process 20-50 samples in a week
- Cost under $100 a sample
- Flexibility to add new genes

RT-PCR Card vs. Microarray



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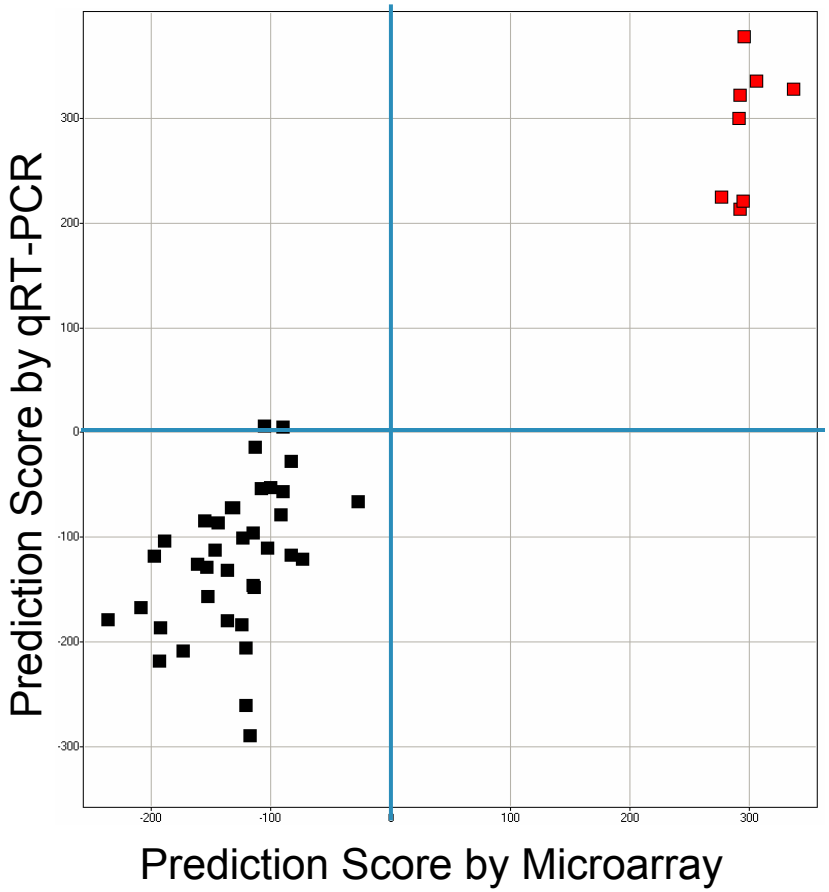
Prediction of AhR Activator

Training Set



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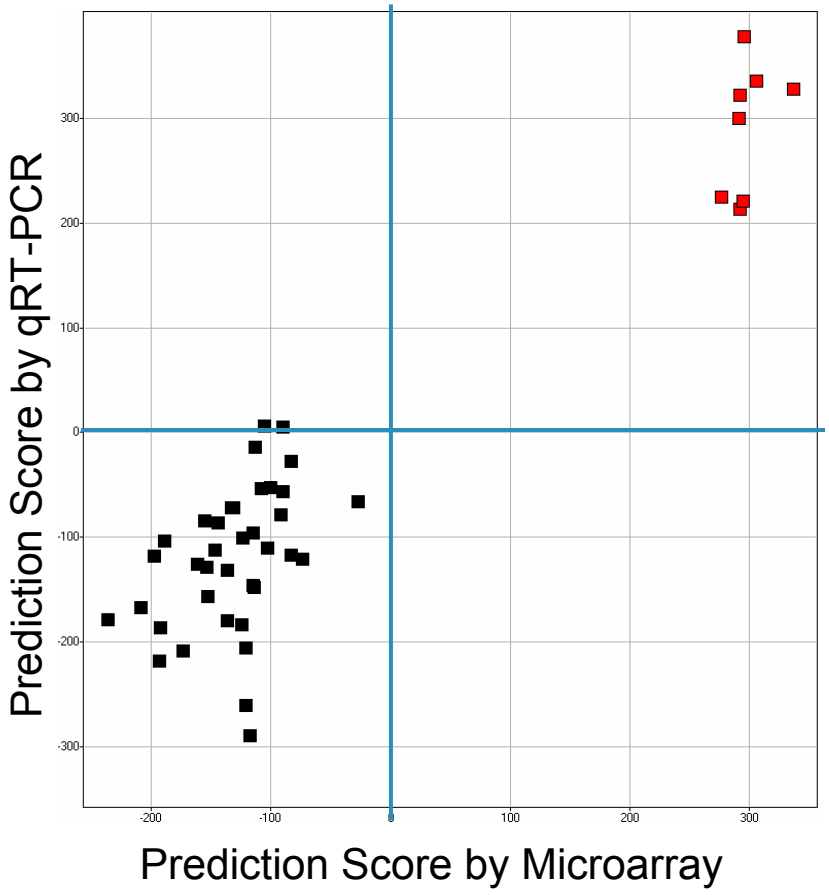
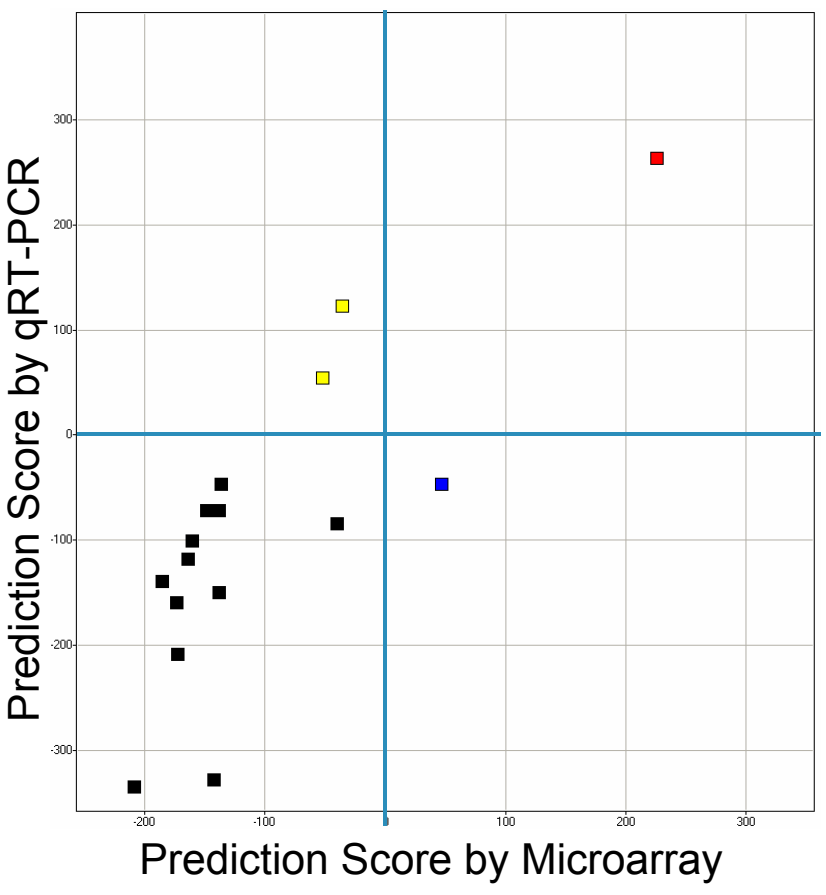


Validation Set



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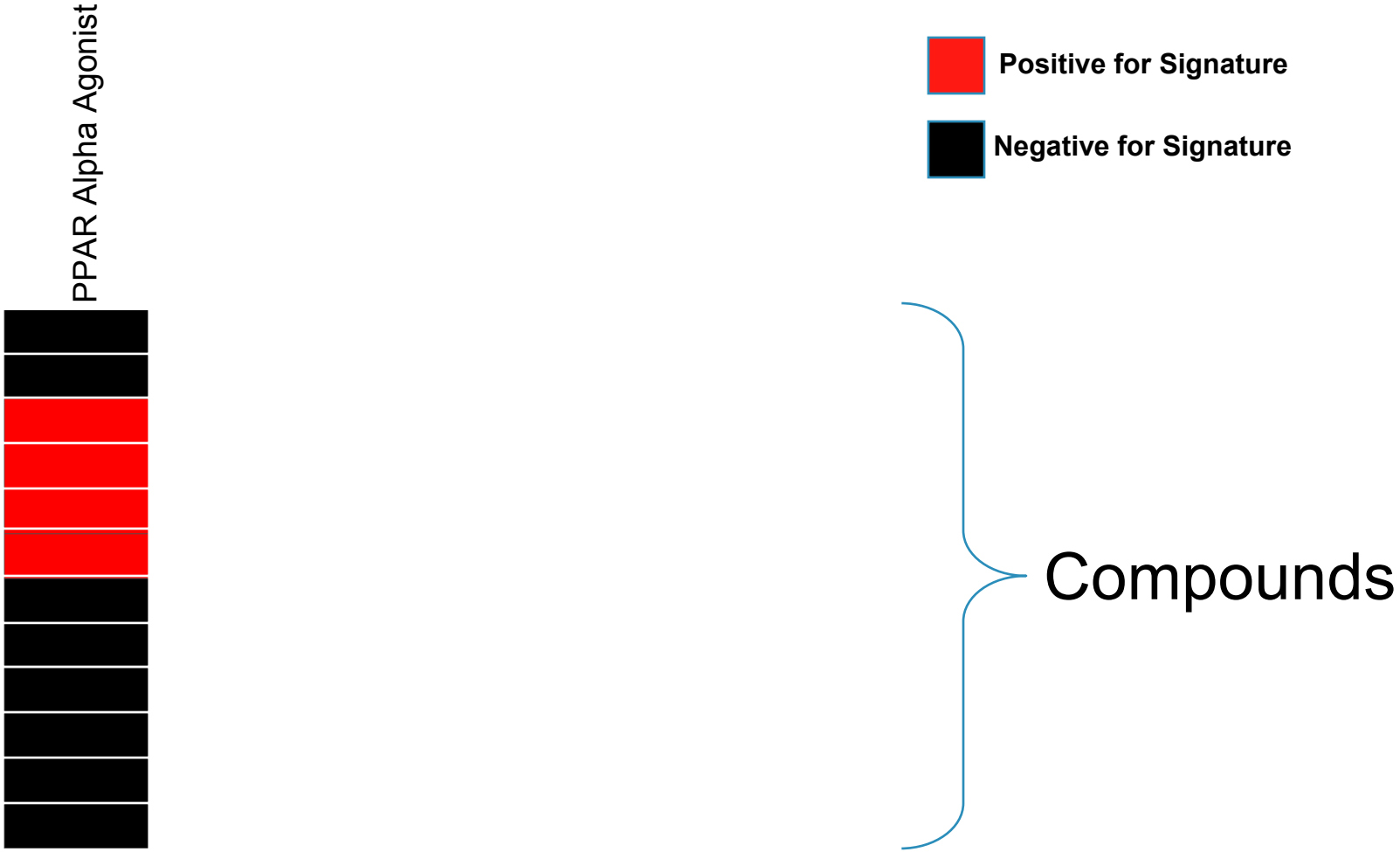
Evaluation of Compounds Using *In Vitro* Toxicogenomics



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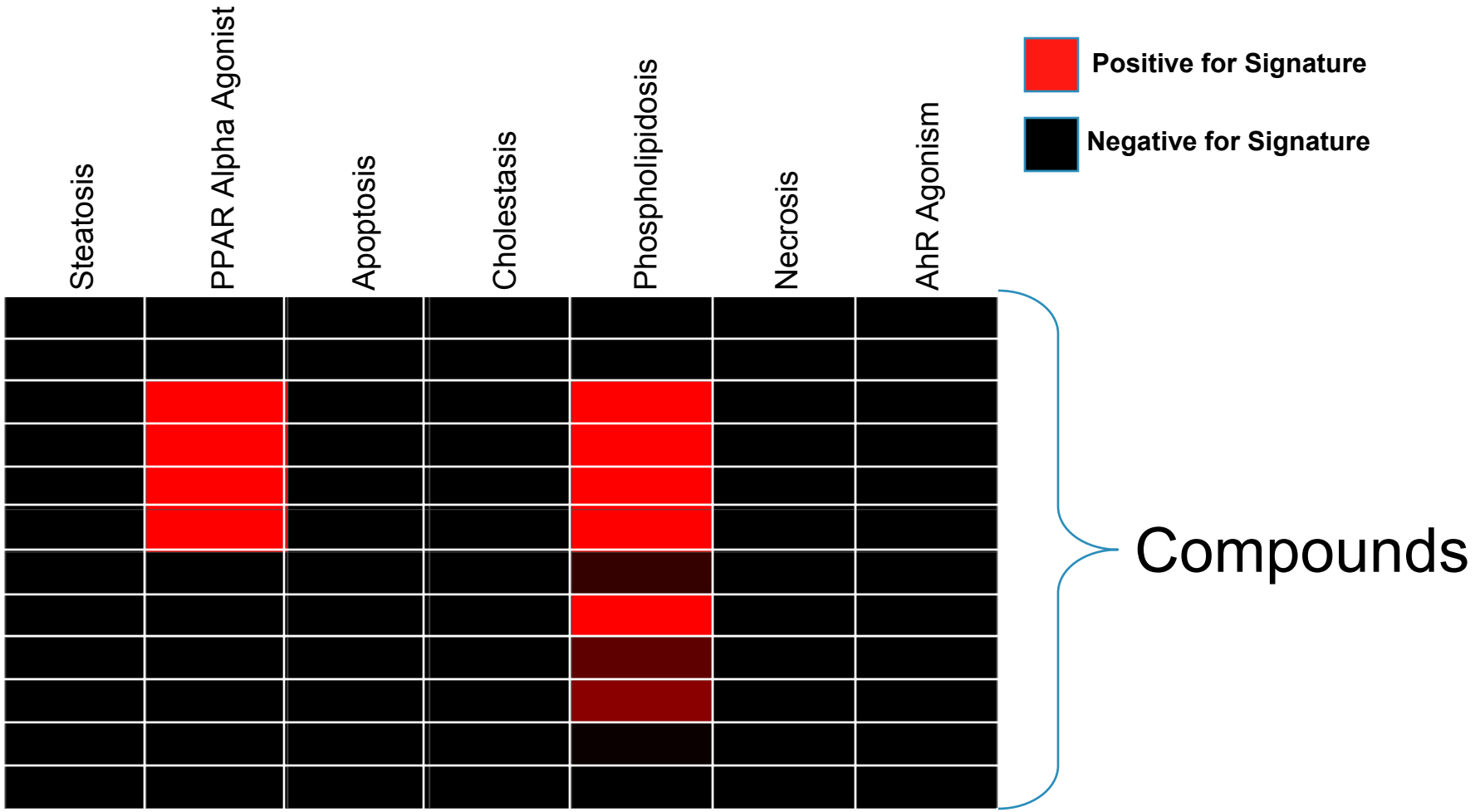

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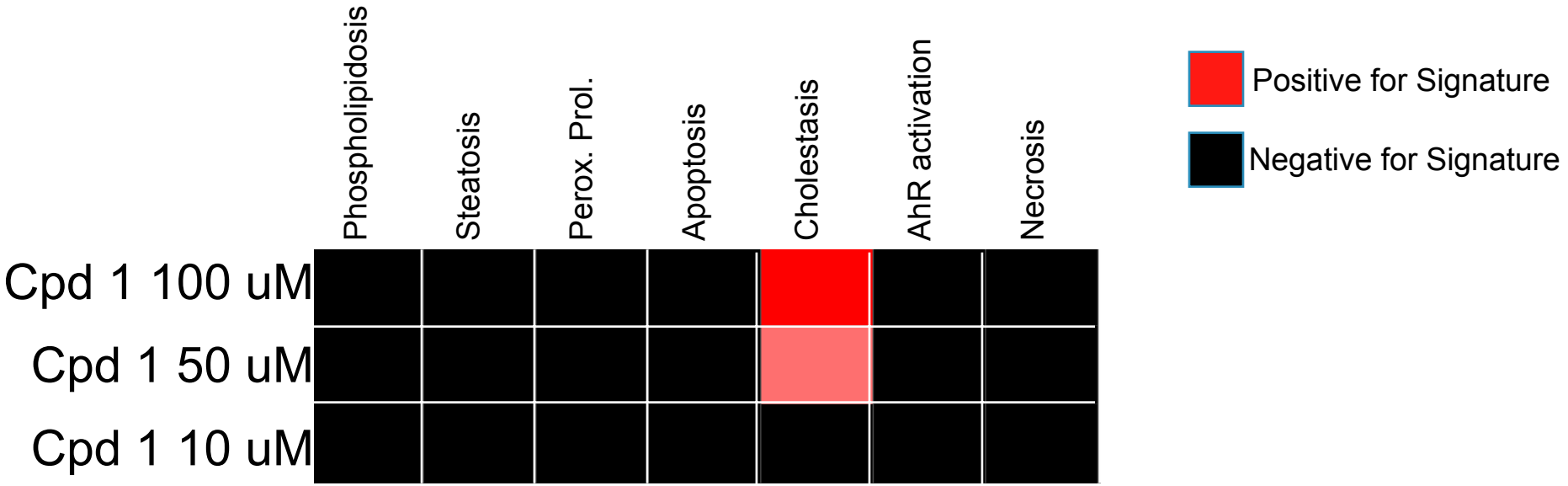
Can Safety Margins Be Determined?



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Human PBMCs for In Vitro Characterization

- Identify toxicities that may be more relevant for humans
- Human PBMCs would reflect genetic diversity present in human population
- Identify biomarkers that can be readily transferred to the clinic

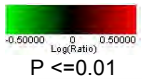
In Vitro Screening Using Human PBMCs

Compound_Name	Dose			Classification	Structure_Activity
	uM	MTD (TC20)	MFD		
DOXORUBICIN	3.59	Yes		DNA damage	DNA intercalator, anthracycline
CARBOPLATIN	1456.5	Yes			DNA-alkylator, platin
CISPLATIN	152.6	Yes			DNA-alkylator, platin
OXALIPLATIN	38.6	Yes			DNA-alkylator, platin
ETOPOSIDE	56.6	Yes		Anti-neoplastic	DNA topoisomerase II inhibitor
ACETAMINOPHEN	6509.2	Yes		Anti-inflammatory	NSAID, COX-3, acetaminophen like
PREDNISOLONE	400		Yes	Immunosuppression	Glucocorticoid and mineralocorticoid receptor agonist
CORTISONE	80		Yes		Glucocorticoid receptor agonist
DEXAMETHASONE	400	Yes	Yes		Glucocorticoid receptor agonist
CYCLOSPORIN A	8.58	Yes			Inhibits T-cell activation
CHLORPROMAZINE	25	Yes		Phospholipidosis	Dopamine receptor antagonist (D), phenothiazine
RIFAMPIN	80.25	Yes		PXR activator	RNA polymerase inhibitor
CLOTRIMAZOLE	17.6	Yes			Sterol 14-demethylase inhibitor
BENZO[A]PYRENE	80		Yes	AhR Agonist	Toxicant, Ah receptor agonist

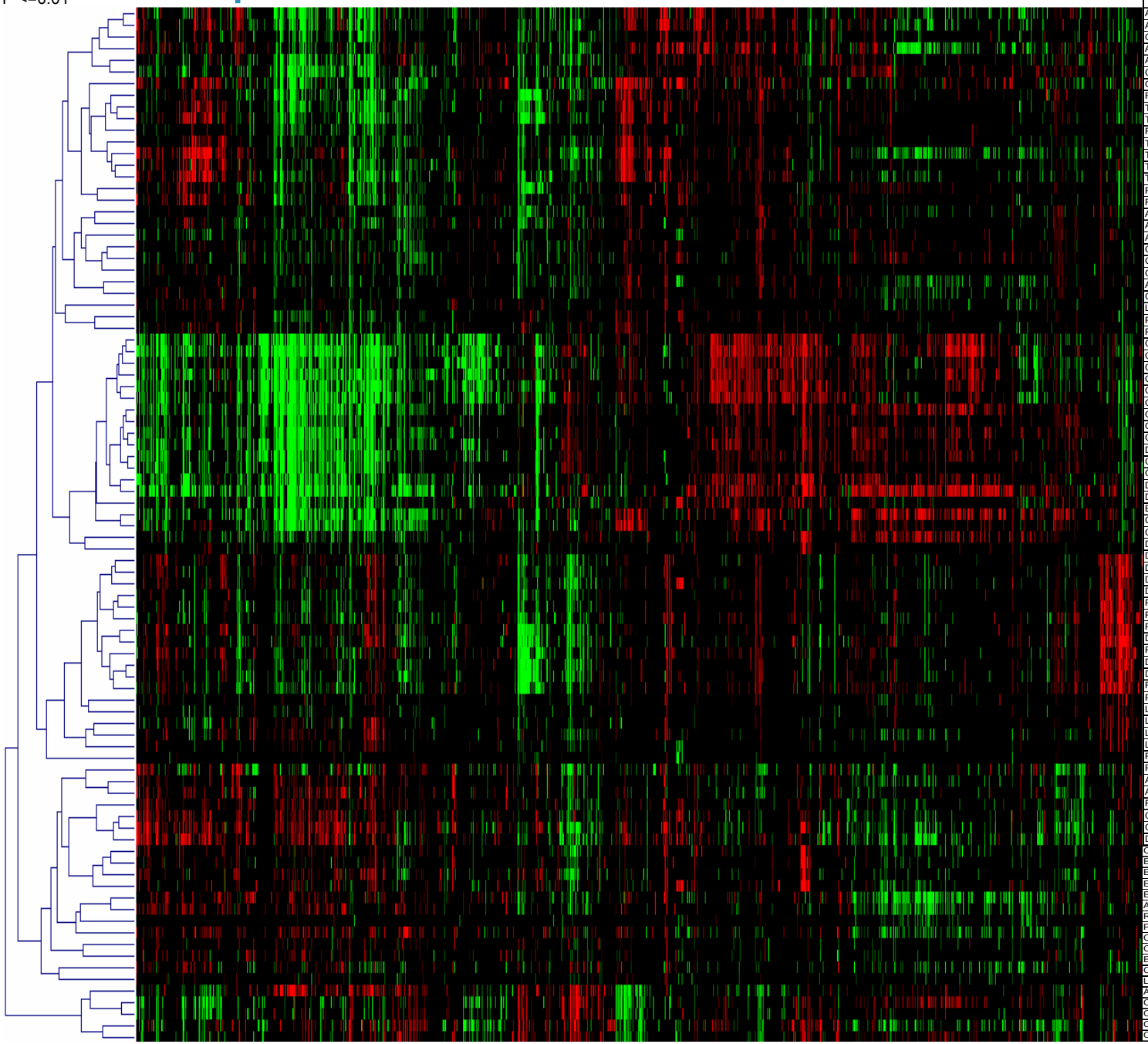
In Vitro Screening Using Human PBMCs

Compound_Name	Dose			Classification
	uM	MTD (TC20)	MFD	
DOXORUBICIN	3.59	Yes		DNA damage
CARBOPLATIN	1456.5	Yes		
CISPLATIN	152.6	Yes		
OXALIPLATIN	38.6	Yes		
ETOPOSIDE	56.6	Yes		Anti-neoplastic
ACETAMINOPHEN	6509.2	Yes		Anti-inflammatory
PREDNISOLONE	400		Yes	Immunosuppression
CORTISONE	80		Yes	
DEXAMETHASONE	400	Yes	Yes	
CYCLOSPORIN A	8.58	Yes		
CHLORPROMAZINE	25	Yes		Phospholipidosis
RIFAMPIN	80.25	Yes		PXR activator
CLOTRIMAZOLE	17.6	Yes		
BENZO[A]PYRENE	80		Yes	AhR Agonist

15 Cpds, 5~6 Donors (FC>=2 and p<=0.01 in at least one experiment, n=14,628)



P <=0.01



Treatment	Classification
ACETAMINOPHEN_5685uM	Anti-inflammatory
ACETAMINOPHEN_5685uM	Anti-inflammatory
OXALIPLATIN_41.66uM	DNA damage
ACETAMINOPHEN_5685uM	Anti-inflammatory
ACETAMINOPHEN_5685uM	Anti-inflammatory
CISPLATIN_112uM	DNA damage
OXALIPLATIN_41.66uM	DNA damage
RAPAMYCIN_13.28uM	Immunosuppression
TROGLITAZONE_94.5uM	PXR activator
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RAPAMYCIN_13.28uM	Immunosuppression
TROGLITAZONE_94.5uM	PXR activator
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TROGLITAZONE_94.5uM	PXR activator
RAPAMYCIN_13.28uM	Immunosuppression
RAPAMYCIN_13.28uM	Immunosuppression
AMITRIPTYLINE_32uM	Phospholipidosis
AMITRIPTYLINE_32uM	Phospholipidosis
AMITRIPTYLINE_32uM	Phospholipidosis
CHLORPROMAZINE_16.14uM	Phospholipidosis
CHLORPROMAZINE_16.14uM	Phospholipidosis
CHLORPROMAZINE_16.14uM	Phospholipidosis
AMITRIPTYLINE_32uM	Phospholipidosis
CHLORPROMAZINE_16.14uM	Phospholipidosis
LOVASTATIN_40uM	PXR activator
PACLITAXEL_37.6uM	PXR activator
PACLITAXEL_37.6uM	PXR activator
CARBOPLATIN_1712.23uM	DNA damage
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CARBOPLATIN_1712.23uM	DNA damage
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CARBOPLATIN_1712.23uM	DNA damage
CARBOPLATIN_1712.23uM	DNA damage
CISPLATIN_112uM	DNA damage
OXALIPLATIN_41.66uM	DNA damage
CYCLOSPORIN A_10.8uM	Immunosuppression
DOXORUBICIN_2.12uM	DNA damage
CISPLATIN_112uM	DNA damage
OXALIPLATIN_41.66uM	DNA damage
DOXORUBICIN_2.12uM	DNA damage
DOXORUBICIN_2.12uM	DNA damage
ETOPOSIDE_66.17uM	Anti-neoplastic
CYCLOSPORIN A_10.8uM	Immunosuppression
CYCLOSPORIN A_10.8uM	Immunosuppression
DOXORUBICIN_2.12uM	DNA damage
DOXORUBICIN_2.12uM	DNA damage
DEXAMETHASONE_400uM	Immunosuppression
DEXAMETHASONE_400uM	Immunosuppression
DEXAMETHASONE_400uM	Immunosuppression
PREDNISOLONE_400uM	Immunosuppression
PREDNISOLONE_400uM	Immunosuppression
PREDNISOLONE_400uM	Immunosuppression
DEXAMETHASONE_400uM	Immunosuppression
PREDNISOLONE_400uM	Immunosuppression
DEXAMETHASONE_400uM	Immunosuppression
DEXAMETHASONE_400uM	Immunosuppression
PREDNISOLONE_400uM	Immunosuppression
PREDNISOLONE_400uM	Immunosuppression
LOVASTATIN_40uM	PXR activator
LOVASTATIN_40uM	PXR activator
LOVASTATIN_40uM	PXR activator
LOVASTATIN_40uM	PXR activator
PACLITAXEL_37.6uM	PXR activator
PACLITAXEL_37.6uM	PXR activator
ACETAMINOPHEN_5685uM	Anti-inflammatory
AMITRIPTYLINE_32uM	Phospholipidosis
RAPAMYCIN_13.28uM	Immunosuppression
CHLORPROMAZINE_16.14uM	Phospholipidosis
CISPLATIN_112uM	DNA damage
DOXORUBICIN_2.12uM	DNA damage
CYCLOSPORIN A_10.8uM	Immunosuppression
ETOPOSIDE_66.17uM	Anti-neoplastic
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LOVASTATIN_40uM	PXR activator
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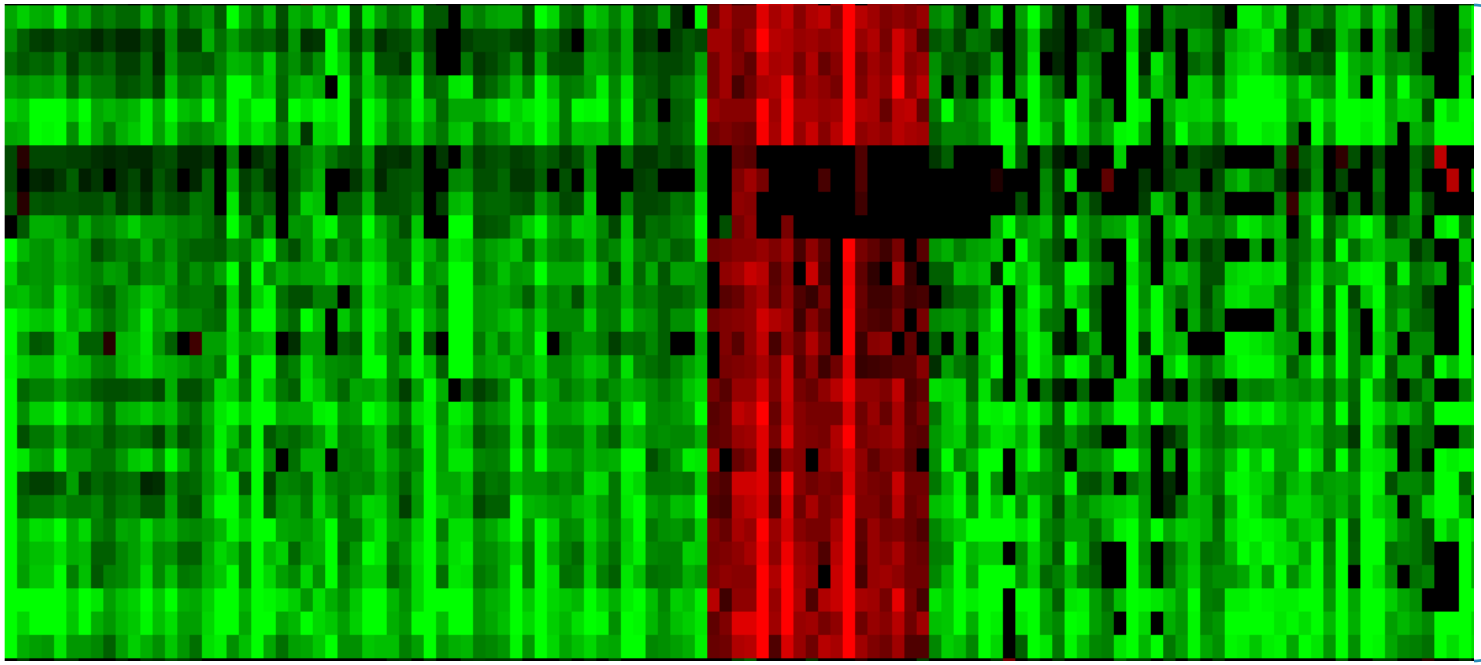
CISPLATIN_112uM	DNA damage
OXALIPLATIN_41.66uM	DNA damage

DNA Damaging Agents Versus Immunosuppresants



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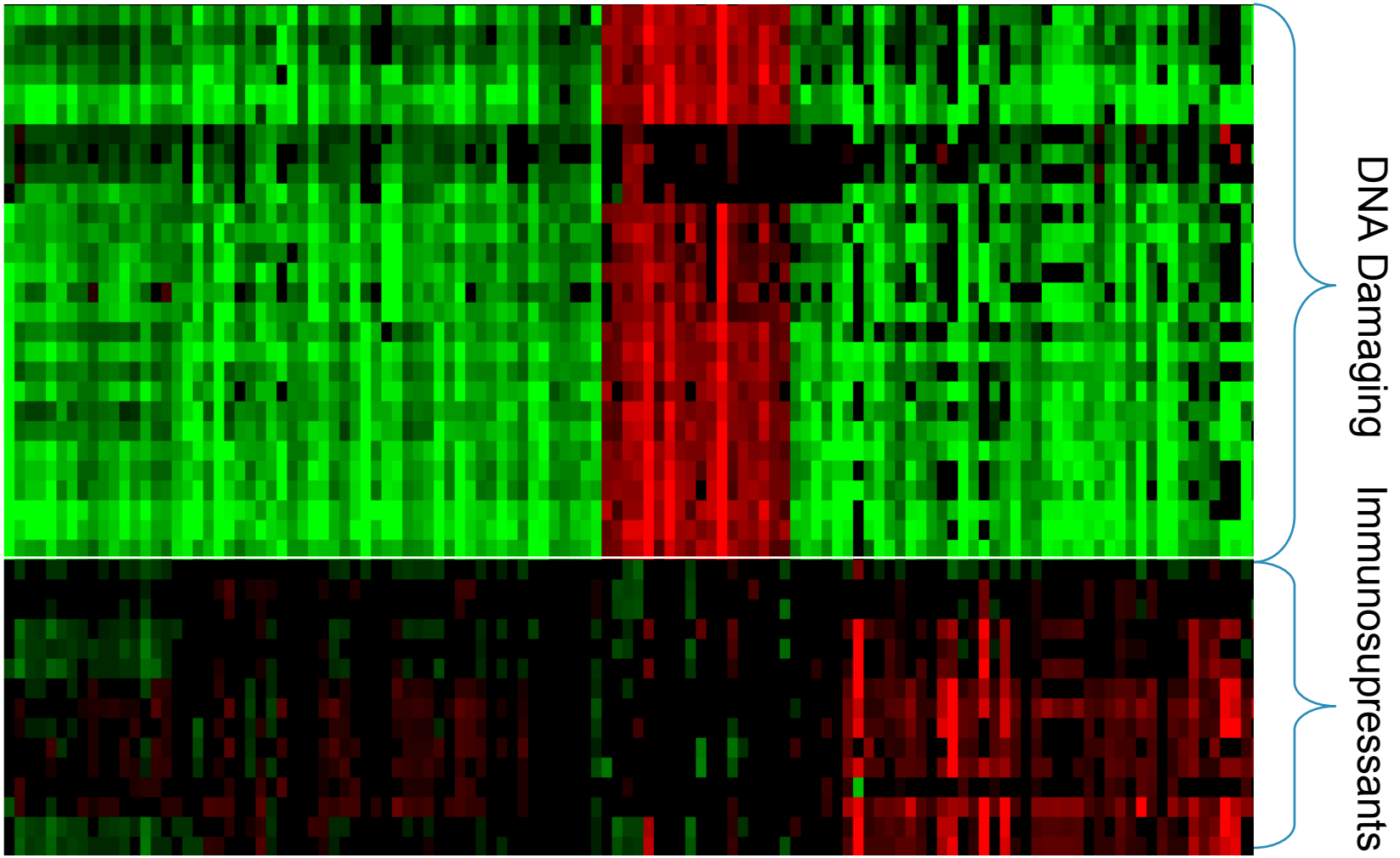
A Promise for Life

DNA Damaging Agents Versus Immunosuppresants



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Summary

1. In vitro toxicogenomics is a useful tool for SAR, prioritization of compounds, selection of backup compounds
2. Limitation is that safety margins in vivo cannot be determined
3. Together with other molecular and cell-based ADMET methods, these efforts should help shift attrition earlier in Drug Discovery

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